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| (54) Title: ANTI-INFLAMMATORY COMPOUNDS | | | | |
| (57) Abstract This invention relates to the novel compounds and pharmaceutical compositions of formulas (I) and (II). This invention also relates to a method of treating or reducing inflammation in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound or composition of formula (I) or (II). | | | | |
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-1-

ANTI-INFLAMMATORY COMPOUNDS

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FIELD OF THE INVENTION

This invention relates to pharmaceutical compositions and their use as anti-inflammatory agents in mammals.

10

BACKGROUND OF THE INVENTION

An early event in the response of most inflammatory cells to immunologic activation and other stimuli is the release of newly formed products (mediators) which alter the function and biochemistry of surrounding cells and tissues. The ensuing biological responses, as well as much of the pathogenesis which is attributed to inflammation and allergy, are thought to be dependent on the effects that these newly-formed mediators have on adjacent cells within the inflammatory region.

In the last 20 years, it has become apparent that lipid mediators are among the most potent and important products which are generated during inflammatory reactions. The synthesis of most lipid mediators is initiated by the specific cleavage of complex phospholipid molecules which contain arachidonate at their sn-2 position. Arachidonic acid is predominantly found in the sn-2 position of phospholipids after redistribution by transacylases and its release by sn-2 acylhydrolases from phospholipids represents the rate-limiting step in the formation of eicosanoids (leukotrienes, prostaglandins and thromboxanes) and other hydroxylated fatty acids. As arachidonic acid is released, it is then converted to oxygenated derivatives by at least two enzymatic systems (lipoxygenase and/or cyclooxygenase). Concomitant with arachidonate release, lysophospholipids are formed. One of these lyso phospholipids, 1-alkyl-2-lyso-sn-glycero-3-phosphocholine, is then acetylated to form platelet-activating factor (PAF). Each of the cell types involved in the inflammatory response produce and secrete a unique subset of lipid mediators. The quantities and nature of the metabolites depend on which enzymes and precursor phospholipid pools are available to inflammatory cells.

Once lipid mediators such as PAF and eicosanoids are formed by the aforementioned pathways, they induce signs and symptoms observed in the pathogenesis of various inflammatory disorders. Indeed, the pathophysiological activity of arachidonic acid (and its metabolites) is well known to those skilled in the art. For example, these mediators have been implicated as having an important role in allergy, asthma, anaphylaxis, adult respiratory distress syndrome, reperfusion injury, inflammatory bowel disease, rheumatoid

-2-

arthritis, endotoxic shock, and cardiovascular disease. Aalmon et al., Br. Med. Bull (1978) 43:285-296; Piper et al., Ann. NY Acad. Sci. (1991) 629:112-119; Holtzman, Am. Rev. Respir. Dis. (1991) 143:188-203; Snyder, Am. J. Physiol. Cell Physiol. (1990) 259:C697-C708; Prescott et al., J. Biol. Chem. (1990) 265:17381-17384.

5 Similar to arachidonate products, PAF is a potent proinflammatory mediator produced by a variety of cells. In vitro, PAF stimulates the movement and aggregation of neutrophils and the release therefrom of tissue-damaging enzymes and oxygen radicals. PAF has also been implicated in activation of leukocytes, monocytes, and macrophages. These activities contribute to the actions of PAF as having (pathological) physiological
10 activity in inflammatory and allergic responses. PAF has also been implicated in smooth muscle contraction, pain, edema, hypotensive action, increases in vascular permeability, cardiovascular disorders, asthma, lung edema, endotoxin shock, and adult respiratory distress syndrome. PAF elicits these responses either directly through its own cellular receptor(s) or indirectly by inducing the synthesis of other mediators.

15 Accordingly, a method which antagonizes the production of free arachidonic acid, its metabolites or PAF will have clinical utility in the treatment of a variety of allergic, inflammatory and hypersecretory conditions such as asthma, arthritis, rhinitis, bronchitis and urticaria, as well as reperfusion injury and other disease involving lipid mediators of inflammation. Many published patent applications or issued US patents exist which
20 describe various compounds having utility as PAF or eicosanoid antagonists. Such patents include U.S. Pat. No. 4,788,205, 4,801,598, 4,981,860, 4,992,455, 4,983,592, 5,011,847, 5,019,581 and 5,002,941.

 Phospholipase A₂'s (PLA₂ (EC 3.1.1.4)) are responsible for the liberation of arachidonic acid from the sn-2 position of phospholipid. They are thought to play an
25 important role in the pathogenesis of inflammation and possibly in immunological dysfunction, both as a cell associated enzyme as well as an extracellular soluble enzyme. Low molecular weight, mammalian Type II 14 kDa PLA₂ has been well characterized and is known to exist in both an extracellular form in inflammatory fluids (Kramer et al., J. Biol. Chem., 264:5768-5775 (1989) and in a cell associated form (Kanda et al., Biochemical and
30 Biophysical Research Communications, 163:42-48 (1989) and has been found in a variety of cells and tissues or extracellularly when released in response to antigenic activators or pro-inflammatory mediators such as Interleukin (IL)-1, IL-6 or tumor necrosis factor (TNF). Its presence in such inflammatory fluids, tissue exudates or serum has therefore implicated Type II-14 kDa-PLA₂'s role in inflammation (Vadas, et al., (1985) Life Sci. 36,
35 579-587; and Seilhamer, et al., (1989) J. Biol. Chem. 264, 5335-5338). Recently, the elevated serum levels of PLA₂ activity during an inflammatory insult have been attributed to cytokine induction of acute phase protein release from liver, of which the 14 kDa-PLA₂ is

-3-

suggested to be a part (Crowl, et al., (1991) J. Biol. Chem. 266, 2647-2651). In addition, soluble PLA₂ activity is markedly elevated in the serum and synovial fluid of patients with rheumatoid arthritis (Stefanski et al., J. Biochem. 100:1297-303 (1986). Furthermore, increasing serum PLA₂ levels have been shown to positively correlate with clinical severity (Bomalaski and Clark, Arthritis and Rheumat. 36:190-198 (1993)). Various inhibitors of PLA₂ have been described in publications and in US Patents. See for instance US Patents 4,959,357; 4,933,365; 5,208,223; 5,208,244; Marshall et al., J. Rheumatology 18:1 (1991); Marshall et al., Phospholipase A₂, Ed. Pyu Wong, Plenum Press, NY (1990) pages 169-181; Wilkerson, et al., Eur. J. Med. Chem., 26:667, 1991 and Wilkerson, Antiinflammatory Phospholipase A₂ Inhibitors, Drugs of the Future, Vol. 15, No. 2 p 139-148(1990). Accordingly, as PLA₂ is important in the liberation of arachidonic acid from phospholipid and may also play a role in the generation of PAF via lysophospholipid formation, inhibition of such an enzyme would be useful for the treatment of disease states caused thereby.

There are many novel forms of phospholipase A₂'s which have recently been discovered. For the purposes herein, members of the sn-2 acylhydrolase family of PLA₂'s are divided into low and high molecular weight enzymes be it from mammalian, or non-mammalian sources. Low molecular weight PLA₂'s will generally have a molecular weight in the range of 12,000 to 15,000. High molecular weight will be in the range of 30,000 or 56,000 kDa to 110,000 by SDS electrophoresis analysis.

A high molecular weight, cytosolic 85 kDa PLA₂ has been isolated and cloned from the human monocytic cell line, U937 (Clark et al., Proc. Natl. Acad. Sci., 87:7708-7712, 1990). The cell-associated Type II-14 kDa-PLA₂ in cell lipid metabolism was thought to be the key rate limiting enzyme in lipid mediator formation, until the recent identification of this cell-associated but structurally distinct 85 kDa sn-2 acylhydrolase, (Clark, et al., supra); and Kramer, et al., (1991) J. Biol. Chem. 266, 5268-5272. Like the Type II-14 kDa enzyme, this enzyme is active at neutral pH and Ca²⁺-dependent, but in contrast exhibits a preference for AA in the sn-2 position of phospholipid substrate and migrates from the cytosol to the membrane in a Ca²⁺-dependent manner and is regulated by phosphorylation (Kramer et al., J. Biol. Chem., 266:5268-5272 (1991). The 85 kDa-PLA₂ is also distinct from 14 kDa-PLA₂s and Ca²⁺-independent PLA₂ as demonstrated by different biochemical characteristics such as stability of the 85 kDa-PLA₂ to DTT, instability to heat and the lack of inhibition by a phosphonate phospholipid TSA inhibitor of 14 kDa-PLA₂. In addition, 85 kDa-PLA₂ has been shown to possess a lysophospholipase A₁ activity which is not observed with the 14 kDa-PLA₂s. The 85 kDa enzyme is similar to the myocardial Ca²⁺-independent PLA₂ (Bomalaski and Clark, Arthritis and Rheumat. 36:190-198 (1993)) in that Ca²⁺ is not required for catalysis and DTNB inhibition is observed. However, 85 kDa-PLA₂ is not

-4-

inhibited by the suicide inactivator bromoenol lactone, suggesting that the enzyme is distinct from the myocardial enzyme as well.

These characteristics make the 85 kDa-PLA₂ a candidate for participation in the liberation of AA from phospholipid stores for subsequent metabolism to lipid mediators.

5 Both the cytosolic 85 kDa PLA₂ and a cell associated Type II 14 kDa PLA₂ have been found in the human monocyte, neutrophil and platelet (Marshall and Roshak, *Biochem. Cell Biol.* 71:331-339 (1993)). As noted above, most of the cellular lipid mediators found elevated in a variety of inflammatory fluids are formed in response to non-pancreatic 14 kDa PLA₂ action. Since arachidonate-containing phospholipids are the key precursors for a

1 0 broad range of lipid mediators it would not be surprising that inflammatory cells would treat these phospholipids differently than other fatty acid-containing phospholipids. In particular, there are enzymes which control the amount of arachidonate in different phospholipid pools and these enzymes are tightly regulated to maintain arachidonate homeostasis. The movement of arachidonate into and from all phospholipids was originally thought to be

1 5 exclusively by Coenzyme A-dependent acyl transferase activities. Holub *et al.*, Adv. Lipid Res., 16:1-125 (1978); Lands *et al.*, In *The Enzymes of Biological Membranes*, ed. Martonosi, A., pp. 3-85, Plenum Press, NY, 1976. However, it has now been demonstrated that an enzyme, Coenzyme A-independent transacylase (CoA-IT), is involved in the movement of 20 carbon higher unsaturated fatty acids, particularly arachidonate, into

2 0 particular (1-alkyl- and 1-alkenyl) phospholipid pools. These are the phospholipid pools of arachidonate that are preferentially mobilized during cell activation and utilized for eicosanoid and PAF biosynthesis, respectively.

CoA-IT has a specificity for certain phospholipids as donor and acceptor molecules. The fatty acid transferred is long chained and unsaturated, and almost exclusively

2 5 arachidonate. Other fatty acids such as the 16:0, 18:1 or 18:2 are not moved into the sn-2 position of alkyl and 1-alkenyl phospholipid pools by CoA-IT. The specificity of CoA-IT is in direct contrast to many other CoA-dependent acylation activities which acylate a wide variety of lysophospholipids with no selectivity for arachidonate.

Accordingly, as CoA-IT is involved in arachidonic acid and phospholipid

3 0 metabolism, inhibition of such an enzyme would be useful for the treatment of inflammatory, allergic and hypersecretory conditions or disease states caused thereby. Therefore, a method by which CoA-IT is inhibited will consequently and preferentially decrease the arachidonate content of 1-alkyl- and 1-alkenyl-linked phospholipids and will therefore decrease the production of pro-inflammatory mediators such as free arachidonic

3 5 acid, prostaglandins, leukotriene and PAF during an inflammatory response.

-5-

SUMMARY OF THE INVENTION

This invention relates to the novel pharmaceutical compositions of Formula (I) and Formula (II) comprising a compound of Formula (I) and/or (II), or pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

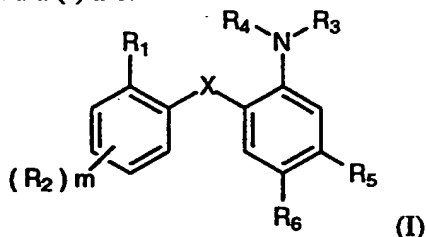
- 5 This invention also relates to a method of treating or reducing inflammation in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound or composition of Formula (I) or (II).

- This invention also relates to a method of treating disease or disorders mediated by lipid inflammatory mediators, free arachidonic acid, its metabolites and/or PAF by administering to a patient in need thereof, an effective amount of a compound of Formula (I) or (II).

This invention also relates to a method of treating disease or disorders mediated by phospholipase A₂ and Coenzyme A independent transacylase by administering to a patient in need thereof, an effective amount of a compound or composition of Formula (I) or (II).

- The present invention also provides for a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound, or pharmaceutically acceptable salt thereof, of Formula (I) or (II).

Compounds of Formula (I) are:



wherein

- 20 R₁ is (CH₂)_nOH or (CH₂)_nCO₂R₈;
 R₈ is hydrogen or C₁₋₄ alkyl;
 n is 0 or an integer having a value of 1;
 X is oxygen or sulfur;
 R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;
 25 m is an integer having a value of 1 or 2;
 R₃ is hydrogen, or optionally substituted C₁₋₈ alkyl, optionally substituted aryl, or (CR₉R₁₀)_pR₁₁;
 p is an integer having a value of 1 to 2,
 R₄ is hydrogen, optionally substituted C₁₋₈ alkyl, or optionally substituted aryl;
 30 R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₇, or (CH₂)_tOH;
 t is 0 or an integer having a value of 1 or 2;
 R₆ is hydrogen or halogen;

-6-

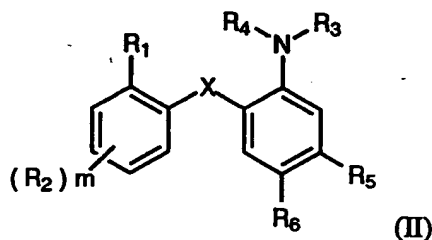
R₇ is hydrogen or C₁₋₄ alkyl;

R₉ and R₁₀ are independently hydrogen, optionally substituted C₁₋₄ alkyl, or optionally substituted aryl;

5 R₁₁ is an optionally substituted alkyl, optionally substituted C₅₋₇ cycloalkyl, or an optionally substituted aryl;
or a pharmaceutically acceptable salt thereof.

Another aspect of the present invention are the compounds represented by the structure having the formula:

10



wherein

R₁ is SO₃H or S(O)_nC₁₋₄ alkyl;

15 n is 0 or an integer having a value of 1 or 2;

X is oxygen or sulfur;

R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;

m is an integer having a value of 1 to 2;

R₃ is hydrogen, or optionally substituted C₁₋₈ alkyl, optionally substituted aryl, or

20 (C(R₉R₁₀)_p R₁₁;

p is an integer having a value of 1 to 2;

R₄ is hydrogen, optionally substituted C₁₋₈ alkyl, or optionally substituted aryl;

R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₇, or (CH₂)_tOH;

t is 0 or an integer having a value of 1 or 2;

25 R₆ is hydrogen or halogen;

R₇ is hydrogen or C₁₋₄ alkyl;

R₉ and R₁₀ are independently hydrogen, optionally substituted C₁₋₄ alkyl, or optionally substituted aryl;

R₁₁ is an optionally substituted C₅₋₇ cycloalkyl, an optionally substituted aryl, or

30 optionally substituted alkyl;

or a pharmaceutically acceptable salt thereof.

-7-

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a novel method of treating inflammatory disease in a mammal in need thereof by administering to said mammal an effective amount of a compound according to Formula (I) or (II). The compounds of Formula (I) and (II) may selectively inhibit the PLA₂ enzyme, the CoA-IT enzyme or both. Inhibition of either or both enzymes will result in the treatment of inflammatory occurrences in mammals.

Inflammatory states in mammals may include, but are not limited to, allergic and asthmatic manifestations, dermatological diseases, inflammatory diseases, collagen diseases, reperfusion injury and stroke. Treatment of both acute and chronic diseases are possible.

Preferred diseases for treatment are arthritis, asthma, allergic rhinitis, inflammatory bowel disease (IBD), psoriasis, reperfusion injury and stroke. For the purposes herein, the compounds of Formula (I) and (II) are preferential and selective inhibitors of the low molecular weight PLA₂ enzyme.

For compounds of Formula (I), R₁ is suitably (CH₂)_nOH or (CH₂)_nCO₂R₈. Preferably R₁ is (CH₂)_nCO₂R₈ and n is preferably 0. R₈ is preferably hydrogen or methyl, more preferably hydrogen, or a pharmaceutically acceptable salt thereof.

Suitably, R₂ is independently a substituent on the benzene ring from 1 to 2 times, and such substituent is selected from hydrogen, halogen, an optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy group. Suitably when R₂ is halogen it is a chlorine or bromine. When R₂ is an optionally substituted C₁₋₈ alkyl, the alkyl is substituted one to three times with halogen, such as fluorine, preferably the alkyl is a methyl substituted by fluorine to yield a trifluoromethyl group. If the alkyl chain is longer, the optionally substituted C₁₋₈ alkyl moiety is preferably a branched C₅ chain, such as 1,1-dimethyl propyl moiety or a C₈ branched chain such as 1,1,3,3-tetramethyl butyl moiety. Suitably m is an integer having a value of 1 or 2.

Suitably, R₃ is hydrogen, optionally substituted C₁₋₈ alkyl, optionally substituted aryl, or (CR₉R₁₀)_p R₁₁, and p is an integer having a value of 1 to 2. When R₃ is an optionally substituted aryl, the ring is preferably phenyl. Preferably R₃ is hydrogen or (CR₉R₁₀)_p R₁₁.

Suitably, R₉ and R₁₀ are independently hydrogen, optionally substituted C₁₋₄ alkyl, or optionally substituted aryl. R₁₁ is preferably an optionally substituted C₅₋₇ cycloalkyl, an optionally substituted aryl, or an optionally substituted alkyl, more preferably an optionally substituted phenyl or cyclohexyl group. Preferably p is 1, and at least one of R₉ and R₁₀ are preferably hydrogen. Most preferably, one of R₉ and R₁₀ are hydrogen and the other an optionally substituted aryl, preferably a phenyl.

Suitably the aryl, aryl alkyl, or alkyl moieties of R₃ are substituted independently,

-8-

one to three times, by halogen, trifluoromethyl, optionally substituted aryl, optionally substituted aryloxy, methoxy, CH₂OH, optionally substituted methyl, or C(O)₂H.

Preferably, the substituent groups are aryl, halogen, or trifluoromethyl. The methyl group is preferably substituted by fluorine to yield a trifluoromethyl group. The substituent

5 halogen groups are preferably Cl, Br and fluorine. When any of the aryl rings herein are optionally substituted it is preferred that the substituents are in the 3,5- position or the 4- position of the phenyl ring. More preferably when the substituent group is an aryl group or an arylalkyl group, it is phenyl or benzyl and the aryl ring is substituted in the 3,5 or 4- positions by halogen, methoxy, CH₂OH, hydroxy, optionally substituted methyl, or
10 C(O)₂H accordingly, such as but not limited to 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-hydroxy, 4-bromo, 4-chloro, or 4-fluoro.

Suitably R₄ is hydrogen, optionally substituted C₁₋₈ alkyl, or optionally substituted aryl. Preferably R₄ is hydrogen, methyl or phenyl, more preferably hydrogen.

Suitably R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₇, or (CH₂)_tOH, and t
15 is 0 or an integer having a value of 1 or 2; R₇ is suitably hydrogen or a C₁₋₄ alkyl group. Preferably when R₅ is CH₂C(O)₂R₇, R₇ is suitably hydrogen or a C₁₋₄ alkyl, preferably hydrogen or t-butyl. Preferred R₅ groups are hydrogen, CF₃, or halogen, such as Cl, or Br. More preferably R₅ is hydrogen, chlorine, or CF₃.

Suitably R₆ is hydrogen or halogen, preferably hydrogen or chlorine.
20

Specifically exemplified compounds of Formula (I) are:

2-[2-[(Diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoic acid;
2-[2-[(Phenylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
2-[2-[[4-Hydroxyphenyl)methyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
25 2-[2-[Cyclohexylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid
2-[2-[[[3,5-Bis(trifluoromethyl)phenyl)methyl]amino]-4-(trifluoromethyl)-
phenoxy]benzoic acid;
2-[2-[(Phenylmethyl)amino]-4,5-dichlorophenoxy]benzoic acid;

30 Preferably compounds of Formula (I) also have the following proviso's:

a) when X is sulfur, and R₁ is (CH₂)_nOH, R₃ and R₄ are both hydrogen, and R₅ is hydrogen, halogen, CF₃ or CH₃; then n is 0; and

b) when X is sulfur, R₁ is (CH₂)_nCO₂R₈, R₈ is C₁₋₄ alkyl, R₃ and R₄ are both hydrogen, and R₅ is hydrogen, halogen, CF₃ or alkyl, then n is 1;

35 c) when X is oxygen, R₁ is (CH₂)_nOH, n is 0, R₄ is hydrogen, and R₅ is hydrogen, halogen, CF₃ or CH₃, then R₃ is other than an unsubstituted phenyl.

-9-

For compounds of Formula (II), R₁ is suitably SO₃H or S(O)_nC₁₋₄ alkyl; and n is a number having a value of 0 to 2. Preferably R₁ is SO₃H, R₂, R₅ and R₆ are preferably the same as for compounds of Formula (I). R₃ and R₄ suitably are the same as for compounds of Formula (I) above are also preferably both hydrogen. In a preferred embodiment, R₁ is SO₃H, R₂ is an optionally substituted C₁₋₈ alkyl, preferably methyl or is a branched C₅ chain, such as 1,1-dimethyl propyl moiety or a C₈ branched chain such as 1,1,3,3-tetramethyl butyl moiety; R₃ and R₄ are preferably hydrogen, R₆ is hydrogen and R₅ is CF₃.

Specifically exemplified compounds of Formula (II) are:

2-[2-Amino-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;

Suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methane sulphonic acid, ethane sulphonic acid, acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid and mandelic acid. In addition, pharmaceutically acceptable salts of compounds of formula (I) may also be formed with a pharmaceutically acceptable cation, for instance, if a substituent R₁ comprises a carboxy group. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations.

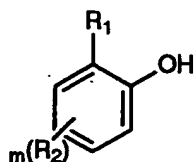
The following terms, as used herein, refer to:

- "halo" - all halogens, that is chloro, fluoro, bromo and iodo;
- "C₁₋₈ alkyl" or "alkyl" - both straight and branched chain radicals of 1 to 8 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and the like;
- "aryl" (on its own or in any combination, such as "aryloxy", or "arylalkyl") means a phenyl and naphthyl ring;
- The term "aralkyl" is used herein to mean an aryl group connected to C₁₋₄ alkyl moiety wherein the alkyl group may be branched or straight as defined above, unless otherwise indicated.

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds are included within the scope of the present invention.

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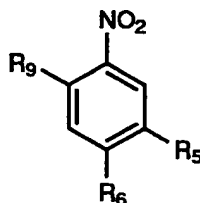
- Compounds of Formula (I) and (II) may be prepared by the process indicated below which comprises reacting a suitably protected compound of Formula (2), which are generally commercially available, and R_1 , R_2 , m are as defined in Formula (I) and/or
- 5 Formula (II). In cases where compounds of Formula (2) are not commercially available, such as where R_1 is $S(O)_3H$, compounds of formula (2) may be prepared by sulfonating an appropriately substituted phenol with a suitable sulfonating reagent, such as fuming sulfuric acid.



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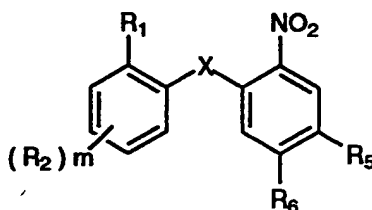
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with a compound of Formula (3), below wherein R_9 is F, Cl, Br or I, and R_5 and R_6 are as in Formula (I):



(3)

- 15 in a suitable solvent such as dimethylformamide and in the presence of a suitable base such as potassium carbonate with or without added copper at a temperature of 25-175°C to provide a compound of Formula (4). In cases where compounds of Formula (2) are not commercially available, such as in some cases when R_1 is RCO_2R_8 , the desired compounds of formula (2) may be prepared by following the procedure of Schmitt *et al.*, *Synthesis*, 1984, 758-760 whose disclosure is incorporated by reference herein.
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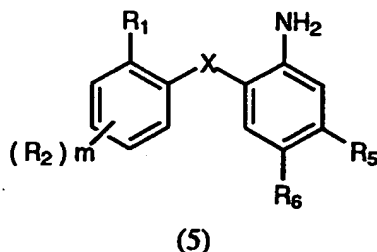


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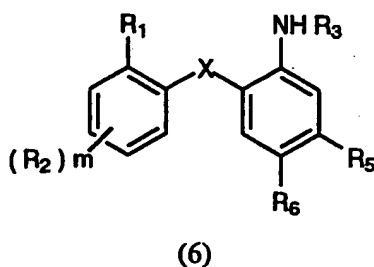
- 25 Reduction of compound (4) with a suitable reducing agent such as iron in a solvent such as acetic acid/ethanol/water, or titanium trichloride in acetic acid water, or H_2 in the

-11-

- presence of a catalyst such as palladium on carbon in a solvent such as ethyl acetate or methanol, provides a compound of Formula (5), which may also after deprotection yield a compound of Formula (I) wherein R₃ and R₄ are hydrogen. Alternatively, compounds of Formula (4) where R₁ is -S(O)₃H may be prepared by sulfonation of a compound of
- 5 Formula (4) where R₁ is H with a suitable reagent such as fuming sulfuric acid.



- 10 Reaction of a compound of Formula (5) with a suitable alkylating agent such as a benzyl halide, diarylmethyl halide, tosylate or mesylate, either neat or in a suitable solvent such as methylene chloride in the presence of a suitable base such as triethylamine or diisopropylethylamine; provides a compound of Formula (6) wherein R₃ is C₁₋₈ alkyl or (CR₉R₁₀)_pR₁₁, and R₄ is hydrogen. In the cases where the alkylating agents are not
- 15 commercially available, they can be prepared by one skilled in the art, for example from the corresponding carboxylic acids or alcohols. Reaction of a compound of formula (6) wherein R₃ is C₁₋₈ alkyl, (CR₉R₁₀)_pR₁₁ and R₄ is hydrogen with a suitable alkylating agent as described above provides a compound of Formula (6) wherein R₃ is C₁₋₈ alkyl or (CR₉R₁₀)_pR₁₁, and R₄ is C₁₋₈ alkyl.
- 20 Alternatively, compounds of Formula (6), wherein R₃ is C₁₋₈ alkyl or (CR₉R₁₀)_pR₁₁, may be prepared by reacting a compound of Formula (5) with an appropriate aldehyde or ketone under dehydrating conditions to form an imine which may be reduced with a suitable reducing agent such as sodium borohydride, sodium cyanoborohydride, or diborane.
- 25 Deprotection (if required) of a compound of Formula (6) and/or conversion to suitable salt forms provides a final compound of Formula (1).



Compounds of Formula (I) wherein R₁ is (CH₂)_n OH may be prepared from compounds of Formula (I) wherein R₈ is hydrogen by reaction with a suitable reducing agent such as borane in a suitable solvent such as tetrahydrofuran.

5

Deprotection (if required) of a compound of Formulas (6) and/or conversion to suitable salt forms provides a final compound of Formula (I).

Without further elaboration, it is believed that one skilled in the art can, using procedures analogous to those described herein, utilize the present invention to its fullest extent. The invention will now be described by reference to the following examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

1 5 SYNTHETIC CHEMISTRY

Temperatures are recorded in degrees centigrade unless otherwise noted.

Example 1

Preparation of 2-[2-[(Phenylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid

a) Methyl 2-[2-nitro-4-(trifluoromethyl)phenoxy]benzoate

2 0 A mixture of methyl salicylate (15.2 g, 0.1 mol), 4-chloro-3-nitrobenzotrifluoride (22.6 g, 0.1 mol), and potassium carbonate (8.3 g, 0.06 mol) in dimethylformamide (100 mL) was stirred under argon and heated in an oil bath to 150°C for 70 min. The reaction mixture was diluted with ethyl acetate (300 mL), filtered, and the residue washed with ethyl acetate. The combined filtrates were evaporated, and the crude product was purified by
2 5 flash chromatography (silica gel, ethyl acetate/hexane) to give the title compound as a pale yellow solid; mp 69-70°C.

b) Methyl 2-[2-amino-4-(trifluoromethyl)phenoxy]benzoate

3 0 A mixture of methyl 2-[2-nitro-4-(trifluoromethyl)phenoxy]benzoate (26 g, 0.076 mol) and 10% palladium on carbon (1 g) in ethyl acetate (400 mL) was hydrogenated in a Parr bottle at 55 psi for 2 h. The reaction mixture was flushed with argon, filtered through Celite® and evaporated. The crude product was purified by flash chromatography (silica gel, ethyl acetate/hexane) to give the title compound as a white solid. ¹H NMR(250 MHz, CDCl₃) δ 7.91 (dd, 1H), 7.45-7.53 (m, 1H), 7.17-7.24 (m, 1H), 6.88-7.03 (m, 3H), 6.78 (d, 1H), 4.25 (br s, 2H), 3.84 (s, 3H).

3 5 c) Methyl 2-[(2-benzylideneamino)-4-(trifluoromethyl)phenoxy]benzoate

Methyl 2-[2-amino-4-(trifluoromethyl)phenoxy]benzoate (500 mg, 0.0016 mol) and

-13-

benzaldehyde (163 mL, 0.0016 mol) were mixed in toluene and heated to 140°C, with a Dean Stark attachment, under argon for 18 h. The solvent was evaporated and the residue flash chromatographed (silica gel, ethyl acetate/hexane) to yield the title compound. ¹H NMR(400 MHz, CDCl₃) δ 8.60 (s, 1H), 7.91 (dd, 1H), 7.81 (d, 2H), 7.35-7.57 (m, 4H), 7.18 (t, 1H), 6.96 (dd, 3H), 3.74 (s, 3H).

d) Methyl 2-[2-[(phenylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoate

Methyl 2-[(2-benzylideneamino)-4-(trifluoromethyl)phenoxy]benzoate (170 mg, 0.0004 mol) was dissolved in methanol at 0°C. Sodium borohydride (6.0 mg, 0.0016 mol) was added slowly to the cold mixture, and stirred under argon for one hour. The mixture was then refluxed for one hour. Methanol was added and the solvents evaporated. The residue was mixed in water and the aqueous phase was extracted with ethyl acetate. The organic phase was dried (MgSO₄) and evaporated. The residue was flash chromatographed (silica gel, ethyl acetate/hexane) to yield the title compound. ¹H NMR(400 MHz, CDCl₃) δ 9.90 (dd, 1H), 7.42-7.50 (m, 1H), 7.17-7.37 (m, 6H), 7.02 (d, 1H), 6.87 (t, 2H), 6.77 (d, 1H), 5.28 (s, 1H), 4.40 (d, 2H), 3.78 (s, 3H).

e) 2-[2-[(Phenylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid

Methyl 2-[2-[(Phenylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoate (70 mg, 0.0002 mol) was dissolved in methanol. 1N Sodium hydroxide (2.4 mL) was added to the mixture and heated to 40°C, under argon, for 2 h. The solvent was evaporated and the residue mixed in water. 3N Hydrochloric acid was added, bringing the pH to 4.0. The aqueous phase was extracted with ethyl acetate. The organic phase was dried (MgSO₄) and evaporated. The residue was flash chromatographed (silica gel, ethyl acetate/hexane/formic acid) to yield the title compound; mp 114°C.

2.5 Example 2

Preparation of 2-[2-[(Cyclohexylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid

a) Methyl 2-[2-(cyclohexylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoate

Methyl 2-[2-amino-4-(trifluoromethyl)phenoxy]benzoate (300 mg, 0.00096 mol) and cyclohexane carboxaldehyde (177 µL, 0.00096 mol) were dissolved in methanol (pH ~4.0) and stirred under argon, at room temperature. Sodium cyanoborohydride (30 mg) was added and mixing continued for several hours. The pH was taken to 2.0 and the methanol was evaporated. The residue was mixed in water and the pH adjusted to 10.0. This mixture was extracted with ethyl acetate (3x) and dried in vacuo. The residue was purified by flash chromatography (silica gel, ethyl acetate/hexane) to yield the title compound. ¹H NMR(400 MHz, CDCl₃) δ 7.95 (d, 1H), 7.45 (t, 1H), 7.20 (t, 1H), 7.0 (d, 1H), 6.80 (m, 3H), 4.85 (s, 1H), 3.70 (s, 3H), 2.95 (d, 2H), 1.80-1.51 (m, 5H), 1.30 (m, 2H), 1.0 (m, 4H).

-14-

b) 2-[2-[(Cyclohexylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid

Following the procedure of Example 1 (e), except substituting tetrahydrofuran for methanol the title compound was prepared. ¹H NMR(400 MHz, CD₃OD) δ 7.90 (dd, 1H), 7.46 (tt, 1H), 7.20 (tt, 1H), 6.94 (d, 1H), 6.82 (t, 3H), 2.98 (d, 2H), 1.50-1.80 (m, 5H), 1.10-1.30 (m, 2H), 0.9-1.0 (m, 4H).

Example 3

Preparation of 2-[2-[[[3,5-Bis (trifluoromethyl)phenyl]methyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid

10 a) Methyl 2-[2-[[[3,5-bis(trifluoromethyl)phenyl]methyl]amino]-4-(trifluoromethyl)-phenoxy]benzoate

Methyl 2-[2-amino-4-(trifluoromethyl)phenoxy]benzoate (100 mg, 0.00032 mol) and 3,5-bis (trifluoromethyl)benzyl bromide were mixed and heated under argon for sixteen hours to yield the title compound which was used in the next step without further

15 purification.

b) 2-[2-[[[3,5-Bis (trifluoromethyl)phenyl]methyl]amino]-4-(trifluoromethyl)-phenoxy]benzoic acid

Following the procedure of Example 1 (e), except substituting tetrahydrofuran for methanol the title compound was prepared; mp 145.0°C.

20

Example 4

Preparation of 2-[2-[(Phenylmethyl)amino]-4,5-dichlorophenoxy]benzoic acid

a) Methyl 2-(2-nitro-4,5-dichlorophenoxy)benzoate

Following the procedure of Example 1(a) except substituting 1,2-dichloro-4-fluoro-5-nitrobenzene for 4-chloro-3-nitrobenzotrifluoride, the title compound was obtained as a yellow crystalline solid. ¹H NMR(400 MHz, CDCl₃) δ 8.12 (s, 1H), 8.06 (dd, 1H), 7.62-7.66 (m, 1H), 7.38-7.42 (m, 1H), 7.17 (dd, 1H), 6.83 (s, 1H), 3.78 (s, 3H).

b) Methyl 2-(2-amino-4,5-dichlorophenoxy)benzoate

A solution of methyl 2-(2-nitro-4,5-dichlorophenoxy)benzoate (2 g, 5.86 mmol) in acetic acid (50 mL) was treated with 20% aqueous titanium trichloride (22 mL, 36 mmol). The solvent was evaporated, and the aqueous residue was made basic with aqueous sodium hydroxide. The crude product was extracted with ethyl acetate, dried over anhydrous sodium sulfate, filtered and evaporated. Flash chromatography (silica gel, ethyl acetate/hexane), followed by recrystallization from ethyl acetate/hexane gave the title compound. ¹H NMR (400 MHz, CDCl₃) δ 7.90 (dd, 1H), 7.46-7.50 (m, 1H), 7.18-7.26 (m, 1H), 6.93 (dd, 1H), 6.89 (s, 1H), 6.83 (s, 1H), 4.08 (br s, 2H), 3.87 (s, 3H).

35

c) 2-[2-[(Phenylmethyl)amino]-4,5-dichlorophenoxy]benzoic acid

-15-

- Following the procedure of Example 1(c-e) except substituting methyl 2-(2-amino-4,5-dichlorophenoxy)benzoate for methyl 2-[2-amino-4-(trifluoromethyl)phenoxy]benzoate, and substituting diborane at room temperature for sodium borohydride at 0°C gave the title compound after recrystallization from ethyl acetate/hexane as a white crystalline solid; mp 180-181°C.

Example 5

Preparation of 2-[2-[(4-Hydroxyphenyl)methyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid

- 1 0 a) 2-[2-[(4-Hydroxyphenyl)methyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid

Following the procedure of Example 1(a-e) except substituting 4-hydroxybenzaldehyde for benzaldehyde, gave the title compound; mp 136°C.

Example 6

- 1 5 Preparation of 2-[2-[(Diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoic acid

a) Methyl 2-[2-[(Diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoate

- Methyl 2-(2-amino-4-trifluoromethylphenoxy)benzoate (0.2 g, 0.66 mmol) was dissolved in triethylamine (2 mL) and stirred under argon. Chlorodiphenylmethane (0.25 mL, 1.4 mmol) was added and the mixture was heated at reflux for 48 h. The solvent was evaporated and the residue was purified by flash chromatography (silica gel, ethyl acetate/hexane) to give the title compound. ¹H NMR (400 MHz, CDCl₃) δ 7.89 (dd, 1H), 7.47-7.51 (m, 1H), 7.19-7.37 (m, 11H), 7.00 (d, 1H), 6.73-6.86 (m, 3H), 5.57 (d, 1H), 5.49 (d, 1H), 3.66 (s, 3H).

b) 2-[2-[(Diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoic acid

- 2 5 Following the procedure of Example 1(e) except substituting methyl 2-[2-[(diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoate for methyl 2-[2-[(Phenyl)methyl]amino]-4-(trifluoromethyl)phenoxy]benzoate the title compound was obtained after recrystallization from ethyl acetate/hexane as a white crystalline solid; mp 148-149°C.

Example 7

2-[2-Amino-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid

a) 2-(2-Nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzene

- 3 5 A mixture of (1,1-dimethylpropyl)phenol (820 mL, 0.005 mol), 3-bromo-3-nitrobenzotrifluoride (1.35 g, 0.005 mol) and potassium carbonate (1.38 g, 0.010 mol) in dimethylformamide (25 mL) was stirred, under argon, and heated in an oil bath to 150°C for 16 h. The reaction mixture was concentrated on rotovapor and the residue was partitioned between ethyl acetate and water. The aqueous was washed with ethyl acetate and the

-16-

organic fractions evaporated. The crude product was flash chromatographed (silica gel, ethyl acetate/hexane) to yield the title compound, a pale yellow solid. ¹H NMR (250 MHz, CDCl₃) δ 8.22 (d, 1H), 7.6. (dd, 1H), 7.37 (m, 2H), 6.97 (dd, 3H), 1.65 (m, 3H), 1.30 (m, 6H), 0.67 (t, 2H).

- 5 b) 2-(2-Nitro-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

2-(2-nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzene was added to fuming sulfuric acid (10 mL) and allowed to stir for 1 h. The reaction mixture was quenched in ice and the aqueous phase was extracted with ethyl acetate. The organic phase
10 was dried (MgSO₄) and evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to yield the title compound, a yellow solid. ¹H NMR (250 MHz, CDCl₃) δ 7.97 (dd, 2H), 7.69 (dd, 1H), 7.43 (dd, 1H), 7.04 (s, 1H), 6.85 (d, 1H), 1.75 (q, 2H), 1.34 (m, 6H), 0.67 (m, 3H).

- 15 c) 2-(2-amino-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt.

2-(2-nitro-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (6.82 g, 0.015 mol) was mixed in ethyl acetate (400 mL) and palladium on carbon catalyst (2.22 g) was added, under argon. The mixture was hydrogenated, in a parr bottle, at 53 psi for two hours. The reaction mixture filtered through Celite®, washed with
20 ethyl acetate /methanol and the solvent evaporated. The crude product was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to yield the ammonium salt, an off-white solid. The ammonium salt was mixed in methanol and water with sodium bicarbonate (361 mg, 0.0043 mol) to yield the title compound. MS (FAB) m/e 448 [M+Na]⁺.

25

METHODS OF TREATMENT

The compounds of Formula (I)/(II), or pharmaceutically acceptable salts thereof, can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of an inflammatory disease state in a mammal, preferably a human.

- 30 Inhibition of PLA₂ and/or CoA-IT and the simultaneous reduction of PAF, free arachidonic acid and eicosanoid release from inflammatory cells according to this invention is of therapeutic benefit in a broad range of diseases or disorders. The invention herein is therefore useful to treat such disease states both in humans and in other mammals.

- 35 Inhibition of CoA-IT and 14 kDa PLA₂ by the compounds of Formula (I) and/or Formula (II) is an effective means for simultaneously reducing PAF, free arachidonic acid and eicosanoids produced in inflammatory cells. The therapeutic utility of blocking lipid mediator generation has been recognized for many years. For example, inhibitors of

-17-

cyclooxygenase, such as aspirin, indomethacin, acetaminophen and ibuprofen, have demonstrated broad therapeutic utilities. CoA-IT inhibitors inhibit cyclooxygenase products. Another class of inhibitors which are used in a broad range of inflammatory disorders are the corticosteroids. Corticosteroids act in a variety of ways, e.g. to induce inflammatory cells to produce proteins which inhibit free arachidonic acid release or to down regulate PLA₂ mRNA formation. Both 14 kDa PLA₂ inhibitors and CoA-IT inhibitors block the release of free arachidonic acid. Inhibitors of 5-lipoxygenase block the production of leukotrienes and leukotriene antagonists prevent the bioactions of leukotrienes. Recent studies indicate that both will have broad therapeutic utilities. Both 14 kDa PLA₂ inhibitors and CoA-IT inhibitors block the production of leukotrienes. Inhibitors of phospholipase A₂ block the release of free arachidonic acid and the formation of lyso PAF (the immediate precursor of PAF). PLA₂ inhibitors are recognized to have broad therapeutic utilities. It does not, however, follow that the disease states noted above must in fact be caused by altered CoA-IT or PLA₂ activity. Thus, the disease state itself may not be directly mediated by CoA-IT or PLA₂ activity. It only follows that CoA-IT or PLA₂ activity is required for the continued expression of symptoms of the disease state and that CoA-IT or PLA₂ inhibitors will be beneficial against the symptoms of these disease states.

Recognition that 14 kDa PLA₂ and/ or CoA-IT inhibitors reduce PAF production has a number of therapeutic implications. PAF itself has been implicated as being involved in a number of medical conditions. Thus in circulatory shock, which is characterized by systemic hypotension, pulmonary hypertension and increased lung vascular permeability, the symptoms can be mimicked by infusion of PAF. This coupled with evidence showing that circulating PAF levels are increased by endotoxin infusion indicate that PAF is a prime mediator in certain forms of shock.

Intravenous infusion of PAF at doses of 20-200 pmol kg⁻¹ min⁻¹ into rats has been reported to result in the formation of extensive haemorrhagic erosions in the gastric mucosa. Thus PAF is the most potent gastric ulcerogen yet described whose endogenous release may underlie or contribute to certain forms of gastric ulceration. Psoriasis is an inflammatory and proliferative disease characterized by skin lesions. PAF is pro-inflammatory and has been isolated from lesioned scale of psoriatic patients indicating PAF has a role in the disease of psoriasis. And finally, increasing evidence supports a potential patho-physiological role for PAF in cardiovascular disease. Thus recent studies in angina patients show PAF is released during atrial pacing. Intracoronary injection of PAF in pigs induces a prolonged decrease in coronary flow and, in guinea pig hearts, it induces regional shunting and ischemia. In addition PAF has been shown to initiate thrombus formation in a mesenteric artery preparation, both when administered exogenously and when released endogenously. More recently PAF has been shown to play a role in brain ischemia induced

in animal models of stroke. Thus the compounds of the invention, by virtue of their ability to antagonize either CoA-IT and/or PLA₂, thus block the production of PAF, free arachidonic acid and its metabolites, are likely to be of value in the treatment of any of the above conditions.

5 The action of a PLA₂ inhibitor can be distinguished from the activity of a CoA-IT inhibitor based on their specific actions on their respective enzymes and by their different effects in cellular assays. For example only CoA-IT inhibitors have the ability to interfere with the mobilization of radiolabelled arachidonic acid to move from the alkyl-PC pool to the alkenyl PE pool. Selective inhibitors of 14 kDa PLA₂ are without an effect in this assay
10 (assay E). Alternatively, CoA-IT inhibitors will inhibit both LTC₄ and PGE₂ release from activated monocytes while selective PLA₂ inhibitors inhibit LTC₄ release but spare prostanoid formation or production (assay F).

Disease states which could benefit from the inhibition of lipid mediator production include, but are not limited to, adult respiratory distress syndrome, asthma, arthritis,
15 reperfusion injury, endotoxic shock, inflammatory bowel disease, allergic rhinitis and various inflammatory skin disorders. Each of these disorders is mediated in some part by lipid mediators of inflammation. Compounds which inhibit CoA-IT, by virtue of their ability to block the generation of lipid mediators of inflammation, are of value in the treatment of any of these conditions. Similarly compounds which inhibit PLA₂, by virtue
20 of their ability to block the generation of lipid mediators of inflammation stemming from activation and/or release of this enzyme are of value in the treatment of these conditions. In particular, an inhibitor of CoA-IT, for instance would offer an advantage over the classical NSAIDs which affect only prostanoid production (and not PAF biosynthesis) thereby inhibiting both the acute and cell-mediated "chronic" inflammatory processes. Further an
25 advantage of the PLA₂ inhibitor would be their affect on human monocyte leukotrienes and PAF formation, while immunosuppressive prostanoids, such as PGE₂, are spared. Likewise, selective inhibition of COX-2, by compounds herein, is useful for the treatment of diseases mediated thereby, such as arthritis, and IBD, for relief of pain, and inflammation, without the gastric and renal side effects associated with COX-1 inhibition.

30 Treatment of disease states caused by these lipid inflammatory mediators i.e., arachidonate, eicosanoids and PAF, include certain cardiovascular disorders such as but not limited to, myocardial infarction, stroke, circulatory shock, or hypotension, ischemia, reperfusion injury; inflammatory diseases such as, but not limited to, arthritis, inflammatory bowel disease, Crohn's disease, or ulcerative colitis; respiratory diseases such as but not
35 limited to, asthma, or adult respiratory distress syndrome: anaphylaxis, shock, such as but not limited to endotoxic shock; topical diseases, such as but not limited to actinic keratosis, psoriasis, or contact dermatitis; or pyresis.

-19-

In order to use a compound of formula (I)/(II) or a pharmaceutically acceptable salt thereof in therapy, it will normally be formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice. This invention, therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of formula (I) and a pharmaceutically acceptable carrier or diluent.

Compounds of formula (I) or (II), pharmaceutically acceptable salts thereof and pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The compounds of formula (I)/(II) may be administered in conventional dosage forms prepared by combining a compound of formula (I) or (II) with standard pharmaceutical carriers according to conventional procedures. Such pharmaceutically acceptable carriers or diluents and methods of making are well known to those of skill in the art, and reference can be found in such texts as Remington's Pharmaceutical Sciences, 18th Ed., Alfonso R. Genarao, Ed., 1990, Mack Publishing Co. and the Handbook of Pharmaceutical Excipients, APhA Publications, 1986.

The compounds of formula (I) or (II) may also be administered in conventional dosages in combination with known second therapeutically active compounds, such as steroids or NSAID's for instance. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg. to about 1g. When a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

Compounds of formula (I)/(II) may be administered topically, that is by non-

-20-

systemic administration. This includes the application of a compound of formula (I)/(II) externally to the epidermis or the buccal cavity and the instillation of such a compound into the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, for instance from 1% to 2% by weight of the formulation. It may however comprise as much as 10% w/w but preferably will comprise less than 5% w/w, more preferably from 0.1% to 1% w/w of the formulation.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 °C. for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic

-21-

technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

- 5 Each dosage unit for oral administration contains preferably from 1 to 250 mg (and for parenteral administration contains preferably from 0.1 to 25 mg) of a compound of the structure (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

- 10 The pharmaceutically acceptable compounds of the invention will normally be administered to a subject in a daily dosage regimen. For an adult patient this may be, for example, an oral dose of between 1 mg and 500 mg, preferably between 1 mg and 250 mg, or an intravenous, subcutaneous, or intramuscular dose of between 0.1 mg and 100 mg, preferably between 0.1 mg and 25 mg, of the compound of the Formula (I)/(II) or a pharmaceutically acceptable salt thereof calculated as the free base, the compound being administered from 1 to 4 times per day.

- 15 The choice of form for administration, as well as effective dosages, will vary depending, inter alia, on the condition being treated. The choice of mode of administration and dosage is within the skill of the art.

20 BIOLOGICAL METHODS:

- To determine activity of the compounds of Formula (I) and (II) various cellular assays can be used to determine in vitro activity. Additionally, various classical in vivo acute inflammatory models which have some aspect of their etiology to elevated eicosanoid levels can be employed, such as the paw edema model, mouse zymosan peritonitis, reverse
- 25 Arthus pleurisy or various skin inflammation assays which are described in Lewis et al., Experimental Models of Inflammation, in the Handbook of Inflammation, Vol. 5, Bonta Ed., Elsevier Science Publishers, NY (1985) whose disclosure is herein incorporated by reference. The TPA induced ear edema model (mouse) as well as the carrageenan paw edema model in the rat are described herein as well. These classical models of
- 30 inflammation will reflect the drug's ability to alter an inflammatory response but cannot address the specificity of drug action. These models have been traditionally designed as non steroid antiinflammatory drug sensitive pharmacological screens and it is important to utilize models which can differentiate PLA2 and CoA-IT inhibitors from NSAIDS.

-22-

Cell-free and Cellular Assessment of Inhibitors

Described herein are several *in vitro* assays both for CoA-IT and PLA₂ enzyme activities. The first employees purified recombinant enzyme or a broken cell assay, assay (a or b, respectively) described below. Alternatively, evaluation of inhibitors can occur in intact cells such as described in the assay, assay (c and d) below. CoA-IT activity can exclusively be measured, and differentiated from PLA₂ inhibition, in intact cells by following the movement of a pulse of [³H] arachidonate as it moves into the 1-alkyl and 1-alkenyl phospholipids in inflammatory cells (assay e). It should be noted for the purposes herein that assays c, d, & f can both be used for PLA₂ and CoA-IT inhibition determination.

Inflammatory Responses *in vivo*

The ability of compounds that inhibit CoA-IT and/or PLA₂ to affect *in vivo* inflammatory responses may be assessed. Inflammatory responses are induced in the mouse ear by the topical application of a pro-inflammatory agent, such as 12-O-tetradecanoylphorbol 13-acetate (assay g). This produces an edematous response, as measured by increases in ear thickness, as well as increased inflammatory cellular infiltrate, as measured by increases in myeloperoxidase activity (as described in the methods). To further validate the mechanism of action inflammation induced by the direct administration of arachidonic acid can be used. In this case compounds altering arachidonic acid mobilization or liberation should be with our effect.

In Vitro Assays

Assay (a): Phospholipase A₂ assay:

Phospholipase A₂ activity of rh Type II- 14 kDa PLA₂ or PLA₂ semi-purified from human synovial joint fluid was measured by the acylhydrolysis of high specific activity (NEN)[³H]-AA-*E. coli* (0.5 mCi/5nmol PL Pi) as previously described in Marshall et al., J. Rheumatology, 18:1, pp59-65 (1991). High specific activity [³H]AA-*E. coli* had up to 95% of the label incorporated into phospholipid which was localized almost exclusively in the sn-2 position, as demonstrated by purified 14kDa PLA₂ or low molecular weight PLA₂ acylhydrolysis and separation of products by thin layer chromatography (TLC) (data not shown). [Predominately used herein was rh Type II 14 kDa PLA₂, or alternatively bovine pancreatic PLA₂ was also be used]. The reaction mixture (50 or 100 ml total volume) contained 25 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM CaCl₂ and [³H]-AA-*E. coli* (low specific activity; 5-8 nmol PL Pi per assay). Assays were incubated for a time

-23-

predetermined to be on the linear portion of a time versus hydrolysis plot. Experiments were conducted with final % hydrolysis values ranging from 2% (400-1000 dpm) to 10% (2000-5000 dpm) acylhydrolysis after blank correction. Reactions were terminated by the addition of 1.0 mL tetrahydrofuran (THF). The whole sample was placed over
 5 aminopropyl solid phase silica columns and eluted with THF:acetic acid (49:1) exclusively separating free fatty acids with greater than 95% recovery. Radiolabel in this eluate was quantitated by liquid scintillation counting. Results were expressed as % of fatty acid hydrolyzed $\left(\frac{\text{[sample dpms - non-specific (blank) dpms]}{\text{total dpms}} \times 100 \right)$ or specific activity which was calculated from hydrolysis values found in the linear portion of time
 10 versus % hydrolysis plots (pmol free fatty acid hydrolyzed/mg/min). Non-specific activity was always less than 1% of the total counts added.

Protein determination

All protein concentrations were determined by Bradford protein analysis kits (Biorad,
 15 Richmond, CA).

Results:

Representative compounds of Formula (I) and (II) which are:

- 2-[2-[(Diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoic acid;
 - 20 2-[2-[(Phenylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
 - 2-[2-[[4-Hydroxyphenyl)methyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
 - 2-[2-[Cyclohexylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid
 - 2-[2-[[[3,5-Bis(trifluoromethyl)phenyl)methyl]amino]-4-(trifluoromethyl)-
 phenoxy]benzoic acid;
 - 25 2-[2-[(Phenylmethyl)amino]-4,5-dichlorophenoxy]benzoic acid; and
 - 2-[2-Amino-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
- demonstrated positive PLA₂ inhibition, generally tested at 50 μm levels.

Assay (b) : CoA-IT Activity

30 The following is a method to measure CoA-IT activity and the effects of compounds on CoA-IT activity. The assay is based upon mixing cellular material containing CoA-IT activity with a stable lyso phospholipid such as 1-alkyl-2-acyl-GPC and measuring the production of phospholipid product such as 1-alkyl-2-acyl-GPC occurring in the absence of added CoA or CoA-fatty acids.

35 Cell Preparation

Any inflammatory cell that contains high levels of CoA-IT activity can be used, such as neutrophils, macrophages or cell lines such as U937 cells. U937 cells were obtained

-24-

from American Type Culture Collection and grown in RPMI-1640 media (Gibco, Grand Island, New York) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at 37°C, 5%CO₂. Cells were grown without differentiation (basal state) by any agent, such as dimethyl sulfoxide. As used herein, "inflammatory cells" include, but are not limited to
5 neutrophils, macrophages, monocytes, lymphocytes, eosinophils, basophils, and mast cells.

Microsomal preparation

Microsomes are prepared using standard techniques. In this case, cells are washed with a buffer of 250 mM sucrose, 10 mM Tris, 1 mM EGTA, 1 mM MgCl₂, pH 7.4 and
1 0 ruptured by N₂ cavitation (750 psi, 10 minutes). The ruptured cells are centrifuged 1000 X g, 5 minutes. The resulting supernatant is centrifuged at 20,000 X g, ~20 minutes. Microsomes are prepared from this supernatant by centrifugation at 100,000 x g, 60 minutes. The resulting pellet is washed once with assay buffer (150 mM NaCl, 10 mM Na₂KPO₄, 1 mM EGTA, pH 7.4), recentrifuged and the pellet resuspended in assay buffer
1 5 (4-20 mg protein/ml) and is stored at -80°C until assayed.

CoA-IT activity

CoA-IT activity is measured in 1.5 ml centrifuge tubes in a total volume of 100 ul. Microsomes are diluted in assay buffer to the desired protein concentration (6-20 ug/tube). The reaction is initiated by addition of [3H] 1-alkyl-2-lyso-sn-glycero-3-phosphocholine
2 0 (GPC) (~0.1 uCi/tube) and 1 µM final cold 1-alkyl-2-lyso-GPC in assay buffer with 0.25 mg/ml fatty acid-poor bovine serumalbumin (BSA) (Calbiochem, La Jolla, CA). [3H]1-alkyl-2-lyso-GPC, approximately 50 Ci/mmol, is from NEN-Dupont (Boston, Massachusetts) and cold 1-alkyl-2-lyso-GPC is from Biomol (Plymouth Meeting, Pennsylvania). Microsomes are pretreated with desired agents for the desired time (10
2 5 minutes) before the addition of [3H]1-alkyl-2-lyso-GPC. The reaction is run for the desired time (10 minutes) at 37°C. The reaction is stopped and the lipids extracted by addition of 100 ul of chloroform:methanol (1:2, v/v) followed by 100 ul of chloroform and 100 ul of 1 M KCl. The samples are vortexed and centrifuged at high speed in a microfuge for 2-3 minutes. An aliquot of the chloroform-extracted materials are separated, usually by TLC in
3 0 chloroform/methanol/acetic acid/water (50:25:8:4, v/v), visualized by radioscanning (Bioscan) and the product, [3H] 1-alkyl-2-acyl-GPC, is scraped and quantified by liquid scintillation spectroscopy. With this TLC system, the synthetic standards of 1-alkyl-2-lyso-GPC and 1-alkyl-2-acyl-GPC are well separated, with R_f values of approximately 0.25 and 0.65, respectively. Other methods can be used to separate substrate from product, including
3 5 but not limited to column chromatography, affinity chromatography and post reaction derivitization. Protein concentration are assessed using the protein assay reagents from Bio-Rad (Richmond, California).

-25-

Results

A variety of compounds have been tested in this assay to determine its selectivity and inability to detect trivial, non-selective inhibitors. Inhibitors of 5-lipoxygenase (5-LO) and cyclooxygenase (CO), such as indomethacin, naproxen, 6-(4'-Fluorophenyl)-5-(4-pyridyl)-2,3-dihydroimidzo-[2,1-b]thiazole and 6-(4'-Fluorophenyl)-5-(4-pyridyl)2,3-dihydroimidzo-[2,1-b]thiazole-dioxide had no effect on CoA-IT activity at concentrations up to 100 μ M. The anti-oxidant BHT also has no effect at concentrations up to 100 μ M. Compounds which complex with phospholipids and inhibit PLA₂ activity, such as quinacrine and aristolochic acid have no effect on CoA-IT activity at concentrations up to 500 μ M. Doxepine, a compound reported to inhibit PAF release did not inhibit CoA-IT at concentrations up to 100 μ M. Sodium diclofenac, reported to decrease leukotriene production by altering arachidonic acid metabolism, had no effect on CoA-IT activity at concentrations up to 500 μ M. These results show that the assay for CoA-IT activity is sensitive and selective.

Assay (c): Arachidonic Acid Release Assay

Preparation of human neutrophils

Human neutrophils are obtained in the laboratory using three different methods. One method uses leukophoresis packs from normal humans and neutrophils are isolated using the histopaque-1077 technique. The blood is centrifuged at 300 x g for 10 minutes. The cell pellets are resuspended in PBS composed of 137 mM NaCl, 8.8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 2.7 mM KCl (Dulbecco's Gibco Laboratories, Long Island, New York) and layered over histopaque-1077 (Sigma, St. Louis, Missouri). The pellets are collected after centrifugation (300 x g for 30 minutes) and washed once in PBS. The cell pellets are exposed briefly to deionized water to lyse any erythrocytes. The remaining cells are collected by centrifugation, suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

The second method isolates human neutrophils from fresh heparinized normal blood using the Histopaque-1077 technique. The blood is layered over Histopaque-1077 (Sigma, St. Louis Missouri) and centrifuged at 400 x g for 30 minutes. The cell pellets are resuspended in 35 ml of PBS and 12 ml of 6% Dextran, followed by Dextran sedimentation at room temperature for 45 minutes. The upper layer is collected and further centrifuged for 10 minutes at 1000 rpm. The cell pellets are exposed briefly to deionized water to lyse erythrocytes. The remaining cells are collected by centrifugation, suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

-26-

The third method isolates human neutrophils from freshly drawn heparinized normal blood using the Percoll technique. The blood is first treated with 6% Dextran at room temperature for a 1 hour sedimentation. The upper layers of plasma are collected and centrifuged at 400 x g for 10 minutes. The cell pellets are resuspended in Percoll 1.070 g/ml supplemented with 5% fetal bovine serum and layered on discontinuous gradients (1.080, 1.085, 1.090, 1.095 g/ml) followed by centrifugation at 400 x g for 45 minutes. The neutrophils are collected from interfaces of 1.080 and 1.085 and the 1.085 and 1.090 Percoll densities, followed by a centrifugation at 400 x g for 45 minutes. The neutrophils are suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

There should be no difference noted in the response of the neutrophils nor in the effects of test compounds in neutrophils isolated by the three different techniques.

Treatment of human neutrophils

Neutrophils are suspended in PBS with 1 mM Ca^{2+} and 1.1 mM Mg^{2+} at concentrations of 5 to 20 x 10⁶ cells per ml. Cells are added to test tubes and treated with the desired compounds for 5 to 10 minutes, then challenged with calcium ionophore A23187, 2 μM , or vehicle control, PBS containing 0.25-1 mg/ml BSA. After 5 to 20 minutes, the reactions are terminated by addition of an equal volume of chloroform:methanol (1:2, v/v) to the samples. [²H₈]Arachidonic acid (50, 100 or 200 ng) is added as an internal standard and the lipids were extracted by addition of equal volumes of chloroform and distilled water. The samples are vortexed and centrifuged at high speed and the chloroform layer removed to a clean tube.

Assay for free arachidonic acid

The chloroform extract for each sample is evaporated to dryness and the material resuspended in hexane. The hexane is passed through a Silica solid phase column (500 mg), washed 2x with hexane and a fatty acid enriched fraction eluted with hexane:ethyl ether (1:1, v/v). Solvents are removed from the samples under a stream of nitrogen then the samples are converted to pentafluorobenzyl esters using pentafluorobenzyl bromide and diisopropylethylamine in acetonitrile. Solvents are removed and samples are suspended in hexane. GC/MS analysis is performed on a suitable instrument, such as a Finnigan MAT TSQ 700 GC/MS/MS/DS (San Jose, California) operated as a single stage quadrupole system or a Hewlett-Packard 5890 with a 5989A MS system.

The peaks corresponding to arachidonic acid and [²H₈]Arachidonic acid are identified and the areas of those peaks compared and the released arachidonic acid calculated as ng of arachidonic acid for each sample. Protein concentrations are assessed using the protein assay reagents from Bio-Rad (Richmond, CA).

-27-

Assay (d): Assay for Production of Platelet-Activating Factor (PAF)**Preparation of human neutrophils:**

5 Blood is obtained from normal humans and neutrophils are isolated as described for the arachidonic acid release assay, above. The final leukocyte preparation should be of greater than 95% purity and viability.

Treatment of human neutrophils

Neutrophils are suspended in PBS at concentrations of 5 to 20 x 10⁶ cells per ml. Cells are added to test tubes and treated with the desired compounds for 5 to 10 minutes, then challenged with calcium ionophore A23187, 2 µM and 20-30 µCi of [3H]acetic acid (NEN-Dupont, Boston, Massachusetts), or the vehicle of PBS with 0.25-1 mg/ml. After 5 to 20 minutes, the reactions are terminated by addition of an equal volume of chloroform:methanol (1:2, v/v) to the samples and the lipids are extracted by addition of equal volumes of chloroform and distilled water. The samples are vortexed and centrifuged at high speed and the chloroform layer removed to a clean tube.

Assay for PAF

The chloroform from each tube is evaporated to dryness and the material suspended in a small volume of chloroform or chloroform:methanol (25-100 µl) and the total material spotted on a Silica TLC plate. The plates are developed in chloroform/methanol/ acetic acid/water (50:25:8:4, v/v) visualized by radioscanning (Bioscan) and the product, [3H]PAF, is scraped and quantified by liquid scintillation spectroscopy. With this TLC system, the R_f value for a synthetic standard of PAF is approximately 0.33.

25 Assay (e): Methods for the evaluation of CoA-IT inhibitors on mobilization of labeled arachidonic acid in intact cells

Measurement of the effect of CoA-IT inhibitors on the transfer of [3H]arachidonate into 1-ether phospholipids in non-stimulated inflammatory cells can be accomplished by general application of the following specific method. Human neutrophils are isolated and resuspended (5 x 10⁷/ml) in Hanks Balanced Salt Solution (HBSS; Gibco). [5,6,8,9,11,12,14,15-³H]-Arachidonic acid (100 Ci/mmol; New England Nuclear) complexed to 200µl HBSS containing 0.25 mg/ml HSA is added to the cell suspension (1 µCi/ml). The cells are incubated with gentle shaking at 37°C for 5 min. The reaction is terminated by the addition of 40 ml ice-cold HBSS containing HSA (0.25 mg/ml). The cells are then removed from the supernatant fluid by centrifugation (225 g, 8 min). Unincorporated [3H]-arachidonic acid is completely removed by two more washes of HBSS containing 0.25 mg/ml HSA. The neutrophils are resuspended in fresh buffer, exposed to various concentrations of a CoA-IT inhibitor or its vehicle and incubated without stimulation

-28-

for 2 hrs. At that time, the tubes containing the cells and buffer are extracted (Bligh & Dyer [Can. J. Biochem. Physiol. (1959) 37, 911-917]) and the phospholipid classes are separated and collected by normal phase HPLC, using a Ultrasphere Silica column (4.6 mm x 250 mm; Rainin) eluted with hexane/2-propanol/ethanol/phosphate buffer (pH 7.4)/acetic acid (490:367:100:30:0.6 v/v) for 5 min at a flow rate of 1 ml/min. The amount of phosphate buffer in the eluting solvent is increased to 5 % over 10 min and this solvent composition is maintained until all the phospholipid classes is eluted from the column (30-40 min) (Chilton, F. H. [Methods Enzymol. (1990)187, 157-166]). The phospholipids are converted into diradylglycerols by addition of phospholipase C, 20 units-40 units of *Bacillus cereus* phospholipase C (Sigma Type XIII) in 100 mM Tris HCl buffer (pH 7.4) for 2.5-6 hr, then converted into 1,2-diradyl-3-acetyl glycerols by incubation with acetic anhydride and pyridine (Chilton, F. H. [Methods Enzymol. (1990)187, 157-166]). The phospholipid subclasses are separated by TLC in benzene/hexane/ethyl ether (50:45:4, v/v), located by image analysis (Bioscan) and the amount of radioactivity in each class is determined by zonal scraping and liquid scintillation counting.

The following is the method for assessing the ability of a compound to alter arachidonate content of cellular phospholipids, which can be generalized for any desired cell. Specifically, mouse bone marrow-derived mast cells are removed from culture and provided with exogenous [³H]arachidonic acid for 30 minutes. The labeled arachidonic acid which had not been incorporated into the cells is then removed by washing the cells 2 times with an albumin-containing buffer. At that point, the cells are treated with various concentrations of CoA-IT inhibitors and then placed back in culture for 24-48 hours. The phospholipids are extracted by the method of Bligh and Dyer [Can. J. Biochem. Physiol. (1959) 37, 911-917] and phospholipids separated by normal phase HPLC by the method of Chilton [Methods Enzymol. (1990)187, 157-166]. The radioactive and mole quantities of arachidonate in complex lipids are determined. At this point, cellular lipid extracts are treated with KOH (0.5 M) to remove fatty acids from complex lipids (phospholipids) and the quantities of arachidonate in these extracts can then be determined by various methods, including gas chromatography and mass spectrometry (Chilton [Methods Enzymol. (1990)187, 157-166]).

Assay (f): Mesurement of stimulated eicosanoid release by human monocytes.

Human Monocyte Isolation. Leukocyte-rich leukopaks obtained from Biological Specialties (Lansdale, PA) are collected from male volunteers who are not taking anti-inflammatory drugs. Leukopaks are centrifuged (90 x g for 15 min) twice to remove the

-29-

platelet-rich plasma. The cell pellet is washed by centrifugation and are resuspended in HBSS without Ca^{2+} or Mg^{2+} . Histopaque 1077 is layered under the cell suspension and centrifuged at $400 \times g$ for 30 min to obtain the buffy coat. The interfacial buffy coat, containing monocytes and lymphocytes, is removed and saved. The buffy coat is washed
5 twice with HBSS without Ca^{2+} or Mg^{2+} by centrifugation. The cell pellet ($4-6 \times 10^8$ cells/30mls) is resuspended in iso-osmotic media (RPMI-1640, 10% heat inactivated fetal bovine serum (FBS), 0.2 mM L-glutamine, 2.5 mM HEPES) and layered over an equal volume of 46% Percoll mixture (10 X PBS/ Percoll; 9.25 / 0.75) and 54% iso-osmotic media and centrifuged for 30 min at $1000 \times g$ (Marshall and Roshak, Biochem. Cell Biol. 71: 331-
10 339, 1993). The monocyte population located at the interface of the Percoll gradient is removed and washed twice in HBSS without Ca^{2+} or Mg^{2+} . This resulted in a greater than 85-90 % pure monocyte population as assessed by differential staining.

Measurement of Stimuli-Induced Eicosanoid Release. Monocytes ($5 \times 10^6/\text{ml}$) are
15 incubated as a suspension in serum-free RPMI-1640 medium containing the vehicle DMSO ($< 1\%$) or drug for 30 min at 27°C after which vehicle or stimuli is added for the indicated time. The stimulating agent is solubilized in DMSO and appropriate vehicle controls are included in all experiments. The amount of stimuli is chosen from the linear portion of a concentration versus product curve usually representing 60-80% maximal stimulation over
20 the indicated incubation time at 37°C (A23187, $1 \mu\text{M}$, (15 min). The reaction is terminated by reduction of pH through addition of citric acid and centrifugation (10 min, $400 \times g$, 4°C). Cell viability is monitored before and after experiments using trypan blue exclusion. The cell-free media is decanted and stored at -70°C until analyzed. Prostaglandin E_2 and LTC_4 are directly measured in cell-free media using enzyme immunoassay (EIA) kits purchased
25 from Caymen Chemical Co. (Ann Arbor, MI). Sample or standard dilutions are made with appropriate media and analyzed in triplicate. Results are obtained by extrapolation from a standard curve prepared in the media and expressed as pg or ng/ml of sample.

Representative compounds of Formula (I) herein which demonstrated positive
30 activity in this assay 2-[2-[(Diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoic acid; which had an IC_{50} of PGE_2 $0.09 \mu\text{M}$ and for LTC_4 and IC_{50} of $0.9 \mu\text{M}$.

In vivo assays

35 Assays (g and h) : Assay (Method) for TPA (assay g) or Arachidonic acid (assay h)-induced Inflammation

Animals: Male Balb/c inbred mice are obtained from Charle River Breeding

-30-

Laboratories (Kingston, NY). Within a single experiment mice (22-25g) are age-matched. These *in vivo* experiments typically involve the use of 5-6 animals/group.

(g) TPA-induced Mouse Ear Inflammation:

5 **Assay of Ear Edema**

TPA (12-O-tetradecanoylphorbol 13-acetate) (Sigma Chemical Company) in acetone (4 mg/20ml) is applied to the inner and outer surfaces of the left ear of BALB/c male mice. The thickness of both ears is then measured with a dial micrometer (Mitutoyo, Japan) at both 2 and 4 hours after treatment, and the data expressed as the change in thickness (10⁻³cm) between treated and untreated ears. The application of acetone does not cause an edematous response; therefore, the difference in ear thickness represents the response to the TPA. After measuring the edema, the inflamed left ears are removed and stored at -70°C until they were assayed for MPO (myeloperoxidase) activity where appropriate.

1 5 **Assay of Myeloperoxidase (MPO) in Inflamed Ear Tissue:**

On the day of the assay, partially thawed ear tissues are minced and then homogenized (10% w/v) with a Tissumizer homogenizer (Tekmar Co.) in 50 mM phosphate buffer (pH 6) containing 0.5% HTAB. The tissue homogenates are taken through three cycles of freeze-thaw, followed by brief sonication (10 sec). The method of Bradley et al. is used with modifications as described. The appearance of a colored product from the MPO-dependent reaction of o-dianisidine (0.167 mg/ml; Sigma) and hydrogen peroxide (0.0005%; Sigma) is measured spectrophotometrically at 460 nm. Supernatant MPO activity is quantified kinetically (change in absorbance measured over 3 min, sampled at 15-sec intervals) using a Beckman DU-7 spectrophotometer and a Kinetics Analysis package (Beckman Instruments, Inc.). One unit of MPO activity is defined as that degrading one micromole of peroxide per minute at 25°C.

Statistics:

3 0 Statistical analysis is done using Student's "t" test. The ED₅₀ are values which cause a 50% inhibition of the inflammatory response and are calculated by regression analysis of the dose response data.

(h) Arachidonic acid induced ear inflammation assay

3 5 Arachidonic acid is dissolved in acetone (1mg/ear) to the left ear of BALB/c male mice. The thickness of both ears is measured with a constant pressure thickness guage 1 hour after treatment and the data expressed as the change in thickness between treated and untreated ears. Test compounds or vehicle are given at the time of AA application. The

-31-

inflammatory cell infiltration is measured by MPO activity as described above in the TPA ear edema assay. After the edema measurements are made, the inflamed ears are removed and assayed for MPO activity.

5 The anti-inflammatory effect of various standard inhibitors topically administered in the AA and TPA induced mouse ear edema model were measured for dexamethasone, scalaradial and Wyeth's compound WY 50,295 at doses of 0.2, 0.1 and 0.3 respectively. The TPA % change in edema was -50 ($p < 0.001$), -46 ($p < 0.01$) and -18 (ns) respectively; for AA the change was -10 (ns), -11 (ns) and -50 ($p < 0.001$). The change in MPO for TPA
10 model was -54 ($p < 0.001$), -65 ($p < 0.001$) and -36 ($p < 0.05$) respectively; for AA it was 0 (ns), -33 (ns) and -90 ($p < 0.001$). One hypothesis is that the AA administration to the ear overrides the need for PLA₂ mediated liberation of substrate for subsequent pro-inflammatory lipid mediator generation or AA mobilization by CoA-IT. In this case an inhibitor of an AA-metabolizing enzyme should be effective while an inhibitor of PLA₂
15 would be ineffective. As noted above, scalaradial and dexamethasone have little or no effect in the AA ear model at concentrations which were effective in the TPA ear model. This can be contrasted to the activity of the selective 5-LO inhibitor WY 50,295 which strongly inhibits inflammation in response to AA. The AA ear model therefore responds well to compounds that exhibit 5-LO inhibitory action and appears to be unaffected by putative
20 PLA₂ inhibitors. This model therefore provides a unique tool with which the contribution of the 5-LO inhibition to the in vivo anti-inflammatory activity of various compounds can be separated from LMW-PLA₂ inhibition.

25 As used herein, various abbreviations and explanations are as follows: [³H], a molecule that contains tritium atoms, a radioactive isotope; A23187, a compound that allows free entry of calcium into a cell; AA, arachidonic acid; arachidonate, arachidonic acid contained within a phospholipid; free arachidonic acid, arachidonic acid that is not contained within a phospholipid; [²H₈]arachidonic acid, the form of arachidonic acid labeled with 8
30 deuterium atoms, a stable isotope; 1-alkyl, 1-Q-alkyl; 1-alkenyl, 1-Q-alk-1'-enyl; BSA, bovine serum albumin; CoA, coenzyme A; CoA-IT, CoA-independent transacylase; DTT, dithiothreitol; EGTA, [ethylenebis(oxyethylenenitrilo)]tetra acetic acid, a calcium chelator; GPC, sn-glycero-3-phosphocholine; EDTA, a metal ion chelator; GPE, sn-glycero-3-phosphoethanolamine; GC/MS, gas chromatography and mass spectrometry; 5HETE, 5(S)-
35 hydroxyeicosa-6,8,11,14-tetraenoic acid; 15HETE, 15(S)-hydroxyeicosa-5,8,11,13-tetraenoic acid; HL-60, American Type Tissue Culture designated cell line similar to a monocyte; LTB₄, leukotriene B₄; LTC₄, leukotriene C₄; LTD₄, leukotriene D₄; lyso PAF,

-32-

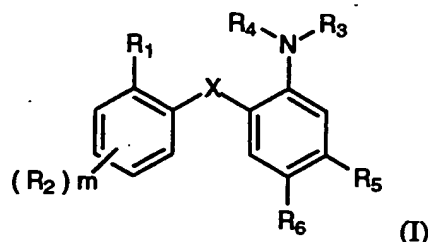
1-alkyl-2-lyso-GPC, lyso platelet-activating factor, PLA₂, phospholipase A₂; PBS, phosphate buffered saline; PAF, platelet activating factor, 1-alkyl-2-acetyl-GPC; PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine, PI, phosphatidylinositol; PMN, polymorphonuclear neutrophilic cell, neutrophil; PS
5 phosphatidylserine; R_f, the distance a compound travels as a fraction of the solvent front; TLC, thin layer chromatography; U937, American Type Tissue Culture designated cell line similar to a monocyte.

The above description fully discloses the invention including preferred embodiments
1 0 thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore, the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the
1 5 invention in which an exclusive property or privilege is claimed are defined as follows.

-33-

What is claimed is:

1. A compound of the formula :



wherein

R₁ is (CH₂)_nOH or (CH₂)_nCO₂R₈;

R₈ is hydrogen or C₁₋₄ alkyl;

10 n is 0 or an integer having a value of 1;

X is oxygen or sulfur;

R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;

m is an integer having a value of 1 or 2;

15 R₃ is hydrogen, or optionally substituted C₁₋₈ alkyl, optionally substituted aryl, optionally substituted heteroaryl, or (CR₉R₁₀)_p R₁₁;

p is an integer having a value of 1 to 2;

R₄ is hydrogen, optionally substituted C₁₋₈ alkyl, or optionally substituted aryl;

R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₇, or (CH₂)_tOH;

t is 0 or an integer having a value of 1 or 2;

20 R₆ is hydrogen or halogen;

R₇ is hydrogen or C₁₋₄ alkyl;

R₉ and R₁₀ are independently hydrogen, optionally substituted C₁₋₄ alkyl, or optionally substituted aryl;

25 R₁₁ is an optionally substituted C₅₋₇ cycloalkyl, an optionally substituted aryl, or optionally substituted alkyl;

provided that

a) when X is sulfur, and R₁ is (CH₂)_nOH, R₃ and R₄ are both hydrogen, and R₅ is hydrogen, halogen, CF₃ or CH₃; then n is 0; and

30 b) when X is sulfur, R₁ is (CH₂)_nCO₂R₈, R₈ is C₁₋₄ alkyl, R₃ and R₄ are both hydrogen, and R₅ is hydrogen, halogen, CF₃ or alkyl, then n is 1;

c) when X is oxygen, R₁ is (CH₂)_nOH, n is 0, R₄ is hydrogen, and R₅ is hydrogen, halogen, CF₃ or CH₃, then R₃ is other than an unsubstituted phenyl;

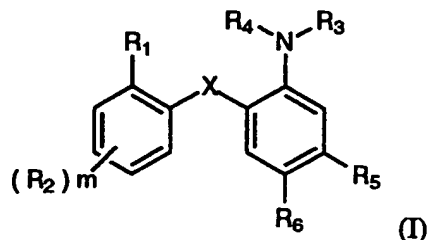
-34-

or a pharmaceutically acceptable salt thereof.

2. The compound according to Claim 1 wherein R_1 is $(CH_2)_nCO_2R_8$.
- 5 3. The compound according to Claim 1 wherein R_4 is hydrogen or phenyl.
4. The compound according to Claim 3 wherein R_3 is $(CR_9R_{10})_p R_{11}$ or phenyl.
5. The compound according to Claim 4 wherein p is 1 and one of R_9 and R_{10} are
10 hydrogen, and the other is an optionally substituted aryl;
6. The compound according to Claim 4 wherein p is 1 both R_9 and R_{10} are hydrogen.
7. The compound according to Claim 4 wherein R_{11} is phenyl, cyclohexyl or
15 4-pyridyl, and p is 1.
8. The compound according to Claim 1 wherein X is oxygen.
9. The compound according to Claim 1 wherein R_5 is hydrogen, halogen, or CF_3 .
- 20 10. The compound according to Claim 9 wherein R_6 is halogen.
11. The compound according to Claim 1 wherein R_2 is hydrogen, halogen, or optionally
substituted C_{1-8} alkyl.
- 25 12. The compound according to Claim 1 which is:
2-[2-[(Diphenylmethyl)amino]-4-trifluoromethylphenoxy]benzoic acid;
2-[2-[(Phenylmethyl)amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
2-[2-[[4-(4-Hydroxyphenyl)methyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
30 2-[2-[Cyclohexylmethyl]amino]-4-(trifluoromethyl)phenoxy]benzoic acid;
2-[2-[[3,5-Bis(trifluoromethyl)phenyl]methyl]amino]-4-(trifluoromethyl)-
phenoxy]benzoic acid; or
2-[2-[(Phenylmethyl)amino]-4,5-dichlorophenoxy]benzoic acid.
- 35 13. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or
carrier and a compound according to Claim 1.

-35-

14. A method for treating an inflammatory disease or disorder in a mammal in need thereof which method comprises administering to said mammal an effective amount of a compound according to the formula :



wherein

R_1 is $(CH_2)_nOH$ or $(CH_2)_nCO_2R_8$;

R_8 is hydrogen or C_{1-4} alkyl;

n is 0 or an integer having a value of 1;

10 X is oxygen or sulfur;

R_2 is hydrogen, halogen, optionally substituted C_{1-8} alkyl, or C_{1-8} alkoxy;

m is an integer having a value of 1 or 2;

R_3 is hydrogen, or optionally substituted C_{1-8} alkyl, optionally substituted aryl, optionally substituted heteroaryl, or $(CR_9R_{10})_p R_{11}$;

15 p is an integer having a value of 1 to 2;

R_4 is hydrogen, optionally substituted C_{1-8} alkyl, or optionally substituted aryl;

R_5 is hydrogen, halogen, CF_3 , CH_3 , $(CH_2)_tC(O)_2R_7$, or $(CH_2)_tOH$;

t is 0 or an integer having a value of 1 or 2;

R_6 is hydrogen or halogen;

20 R_7 is hydrogen or C_{1-4} alkyl;

R_9 and R_{10} are independently hydrogen, optionally substituted C_{1-4} alkyl, or optionally substituted aryl;

R_{11} is an optionally substituted C_{5-7} cycloalkyl, an optionally substituted aryl, or optionally substituted alkyl;

25 or a pharmaceutically acceptable salt thereof.

15. The method according to Claim 14 wherein the inflammatory disease or disorder is allergic rhinitis, ischemia, reperfusion injury, arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, asthma, adult respiratory distress syndrome, 30 anaphylaxis, actinic keratosis, psoriasis, contact dermatitis, or pyresis.

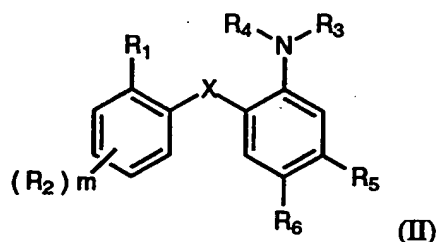
16. The method according to Claim 14 wherein the inflammatory disease or disorder is

-36-

mediated by lipid inflammatory mediators, arachidonic acid, its metabolites and/or platelet activating factor (PAF).

17. The method according to Claim 16 wherein the lipid inflammatory mediators are inhibited by the an inhibitor of the enzyme phospholipase A₂ (PLA₂).

18. A compound having the formula:



10

wherein

R₁ is SO₃H or S(O)_nC₁₋₄ alkyl;

n is 0 or an integer having a value of 1 or 2;

X is oxygen or sulfur;

- 15 R₂ is hydrogen, halogen, optionally substituted C₁₋₈ alkyl, or C₁₋₈ alkoxy;

m is an integer having a value of 1 or 2;

R₃ is hydrogen, or optionally substituted C₁₋₈ alkyl, optionally substituted aryl, or (CR₉R₁₀)_p R₁₁;

p is an integer having a value of 1 to 2;

- 20 R₄ is hydrogen, optionally substituted C₁₋₈ alkyl, or optionally substituted aryl;

R₅ is hydrogen, halogen, CF₃, CH₃, (CH₂)_tC(O)₂R₇, or (CH₂)_tOH;

t is 0 or an integer having a value of 1 or 2;

R₆ is hydrogen or halogen;

R₇ is hydrogen or C₁₋₄ alkyl;

- 25 R₉ and R₁₀ are independently hydrogen, optionally substituted C₁₋₄ alkyl, or optionally substituted aryl;

R₁₁ is an optionally substituted C₅₋₇ cycloalkyl, an optionally substituted aryl, or optionally substituted alkyl;

or a pharmaceutically acceptable salt thereof.

30

19. The compound according to Claim 18 which is:

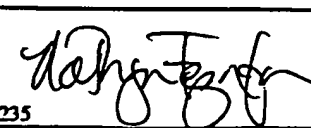
2-[2-Amino-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid.

-37-

20. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a compound according to Claim 18.
- 5 21. A method for treating an inflammatory disease or disorder in a mammal in need thereof which method comprises administering to said mammal an effective amount of a compound according to Claim 18.
- 10 22. The method according to Claim 21 wherein the inflammatory disease or disorder is allergic rhinitis, ischemia, reperfusion injury, arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, asthma, adult respiratory distress syndrome, analphylaxis, actinic keratosis, psoriasis, contact dermatitis, or pyresis.
- 15 23. The method according to Claim 22 wherein the inflammatory disease or disorder is mediated by lipid inflammatory mediators, arachidonic acid, its metabolites and/or platelet activating factor (PAF).
24. The method according to Claim 23 wherein the lipid inflammatory mediators are inhibited by the an inhibitor of the enzyme phospholipase A₂ (PLA₂).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07019

| A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : Please See Extra Sheet. US CL : Please See Extra Sheet. According to International Patent Classification (IPC) or to both national classification and IPC | | | | | | | | | | | | | | | | | | | | |
|---|---|--|--|---|---|--|----|--|--|----|--|---|----|---|--|--|--|--|--|--|
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 560/9,12; 562/30, 45, 46, 47; 564/201; 514/506, 532, 533, 579, 613, 615, 618 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN,CAS | | | | | | | | | | | | | | | | | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | | | | | | | | | | | | | | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | | | | | | | | | | | | | | | | | |
| Y | Chemical Abstracts, Volume 56, issued 1968; K. L. Olivier, "Conversion of aryl ethers into phenols and substituted aromatic amines", columns 1395-1396, DE, 1,100,647, see entire Abstract. | 1, 3-4, 8-9, 11 and 13 | | | | | | | | | | | | | | | | | | |
| Y | US, A, 3,755,449 (ITO ET AL) 28 AUGUST 1973 column 1, lines 30-55. | 1-3, 8-9 and 11 | | | | | | | | | | | | | | | | | | |
| Y | US, A, 3,927,093 (YALE) 16 DECEMBER 1975, columns 1-2. | 1-3, 9-11 and 13 | | | | | | | | | | | | | | | | | | |
| Y | US, A, 3,646,200 (SHEN ET AL) 29 FEBRUARY 1972, columns 1-2. | 1-3, 8-11 and 13 | | | | | | | | | | | | | | | | | | |
| X | | 1-3, 8-11 and 13 | | | | | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | | | | | | | | | | | | | | | | | | | |
| <table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>T</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A* document defining the general state of the art which is not considered to be of particular relevance</td> <td>X*</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*E* earlier document published on or after the international filing date</td> <td>Y*</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>A*</td> <td>document member of the same patent family</td> </tr> <tr> <td>*O* document referring to an oral disclosure, use, exhibition or other means</td> <td></td> <td></td> </tr> <tr> <td>*P* document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table> | | | * Special categories of cited documents: | T | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | *A* document defining the general state of the art which is not considered to be of particular relevance | X* | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | *E* earlier document published on or after the international filing date | Y* | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | A* | document member of the same patent family | *O* document referring to an oral disclosure, use, exhibition or other means | | | *P* document published prior to the international filing date but later than the priority date claimed | | |
| * Special categories of cited documents: | T | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | | | | | | | | | | | | | | | | | | |
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| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | A* | document member of the same patent family | | | | | | | | | | | | | | | | | | |
| *O* document referring to an oral disclosure, use, exhibition or other means | | | | | | | | | | | | | | | | | | | | |
| *P* document published prior to the international filing date but later than the priority date claimed | | | | | | | | | | | | | | | | | | | | |
| Date of the actual completion of the international search 24 AUGUST 1995 | | Date of mailing of the international search report 25 SEP 1995 | | | | | | | | | | | | | | | | | | |
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230 | | Authorized officer JOSEPH M. CONRAD III  Telephone No. (703) 308-1235 | | | | | | | | | | | | | | | | | | |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/07019

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-------------|--|---|
| Y — X | US, A, -3,997,401 (JIU ET AL) 14 December 1976, columns 1-2, 6 and 8. | 1, 3, 8-9, 11 and 13 ----- 1, 3, 8-9, 11 and 13 |
| X | US, A, 3,629,477 (MODEL ET AL) 21 DECEMBER 1971, columns 1-2. | 1, 3, 8-11 and 13 |
| X | JP, A, 58-208278 (CHUGAI PHARM. CO.), 03 DECEMBER 1983, columns 1-2. | 1-3, 8-9, 11 and 13 |
| X | JP, A, 57-2278 ((CHUGAI PHARM. CO.) 07 JANUARY 1982, column 2. | 1-3, 8-9, 11 and 13 |
| A | WO, A, 92/00285 (NIPPON SHINYAKU CO., LTD.) 07 JANUARY 1992, abstract | 1-24 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/07019

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07C 321/24, 211/43, 307/02, 309/31, 233/64; A61K 31/21, 31/235, 31/13, 31/16, 31/165

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

560/9,12; 562/30, 45, 46, 47; 564/201; 514/506, 532, 533, 579, 613, 615, 618

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